MOLECULAR BIOLOGY

SEARCHING FOR PROMOTERS IN THE *secA2* LOCUS OF *MYCOBACTERIUM TUBERCULOSIS*

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Abstract

The enzymatic action of the SecA ATPase plays a vital role in the Sec protein complex, a multimeric (SecA, B, E, F, G, Y) machine for the export and integration of cytosolic and membrane bound proteins into and across the plasma membrane. *Mycobacterium tuberculosis* is unusual in that it possesses two nonredundent *secA* homologues (*secA1 and secA2)*. Preliminary results from the Braunstein laboratory have demonstrated that SecA2 is required for the virulence of *M. tuberculosis* in the mouse model of tuberculosis. The goal of my project was to identify and characterize the initiation and transcription of the *secA2* gene, contained in a 13Kb DNA fragment, isolated from *M. tuberculosis*. To do this a truncated LacZ promoter-trap vector (pCV77) containing an origin of replication for *Escherichia coli* and *M. tuberculosis* was used to analyze the 13 Kb DNA fragment, which contained the *secA2* locus and several downstream as well as upstream genes (2 upstream, and 3 downstream) thought to be contained in one operon. The 13Kb fragment was partially digested with Sau3A, ligated into pCV77 and used to construct a library, which was screened in both *E. coli* and *Mycobacterium smegmatis.* DNA fragments that promoted expression of the truncated and promoterless *lacZ* in pCV77 were then identified as blue colonies on agar plates containing the chromogenic substrate of LacZ. Each bacterial system provided unique working conditions, while *E. coli* was used for its short doubling time and ease of manipulation, the slower growing saprophytic *M. smegmatis* provided a mycobacterial promoter recognition system that *E. coli* could not. Both bacterial species exhibited preliminary positive results, demonstrating that promoters, or promoter like elements may exists in the 13Kb fragment, although further testing is required to definitively identify the promoters on the 13Kb fragment. Further analysis of this library in pCV77, with a complexity of 1480 individual plasmids, will help us achieve our goal of characterizing the *secA2* promoter and operon structure in *M. tuberculosis*.